

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of:	:	Before the Examiner:
Tour et al.	:	Wong, Edna
Serial No.: 10/738,459	:	Group Art Unit: 1753
Filed: December 17, 2003	:	Conf. No.: 9579
Title: USE OF MICROWAVES TO	:	
CROSSLINK NANOTUBES	:	

APPEAL BRIEF

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

I. REAL PARTY IN INTEREST

The real party interest is Rice University, which is the assignee of the entire right, title and interest in the above-identified patent application.

II. RELATED APPEALS AND INTERFERENCES

There are no other appeals or interferences known to Appellants, Appellants' legal representative or assignee which will directly affect or be directly affected by or have a bearing

on the Board's decision in the pending appeal.

III. STATUS OF CLAIMS

Claims 1-27 are pending in the Application. Claims 1-27 are rejected. Claims 1-27 are appealed and are presented in the attached CLAIMS APPENDIX.

IV. STATUS OF AMENDMENTS

Appellants submitted remarks on May 14, 2007 following receipt of the final office action with a mailing date of March 14, 2007. Only remarks, no amendments to the claims, were submitted in this response. In an Advisory Action dated May 24, 2007, the Examiner maintained the rejections of March 14, 2007. A petition under 37 C.F.R. §1.181 disputing the finality of the office action of March 14, 2007 was granted June 14, 2007.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Independent Claim 1:

Claim 1 recites a method for crosslinking carbon nanotubes comprising the steps of (1) *providing* carbon nanotubes; and (2) *irradiating* said nanotubes with microwave radiation to crosslink the carbon nanotubes.

Page 3, paragraph [0008], generally introduces the concept of using microwaves to crosslink carbon nanotubes and the types of products that may be obtained.

Step 1: Page 6, paragraph [0021] describes how to make the *provided* nanotubes and in what form they may be used. Page 6-7, paragraphs [0022] and [0023] describe how the *provided* nanotubes may be modified, both chemically and physically. Page 7, paragraph [0024] and [0025] describes possible states of purity and separation of various nanotube types that may be *provided*.

Step 2: Exemplary *irradiation* conditions are discussed on page 8, paragraph [0026]. Paragraph [0032] gives further details concerning how the *irradiation* may be performed. Pages 8-9, paragraphs [0027]-[0031] detail the nature of possible *crosslinking* motifs that may result from performing the *irradiation*.

Independent Claim 8:

Claim 8 recites a method of *crosslinking* carbon nanotubes comprising (1) *providing* carbon nanotubes; and (2) *irradiating* said carbon nanotubes with microwaves to yield a plurality of *crosslinked* carbon nanotubes, wherein *crosslinking* is generated between the sidewalls of adjacent carbon nanotubes.

See description of Claim 1 above and, for specific discussion concerning sidewall reactions, see page 8, paragraphs [0028] and [0029].

Independent Claim 19:

Claim 19 recites a method of *crosslinking* single-wall carbon nanotubes comprising: (1) *providing* single-wall carbon nanotubes; and (2) *irradiating* said single-wall carbon nanotubes with microwaves to yield a plurality of *crosslinked* single-wall carbon nanotubes; wherein *crosslinking* is generated between the sidewalls of adjacent single-wall carbon nanotubes; and wherein the step of irradiating is carried out in an inert environment such as ultra-high vacuum, high vacuum, inert gases, and combinations thereof.

See description of Claim 8 above and, for specific discussion concerning the reaction environment, see pages 9-10, paragraphs [0033]-[0037].

VI. GROUND OF REJECTION TO BE REVIEWED ON APPEAL

A. Whether Claims 1-4 and 7 are unpatentable under 35 U.S.C. §102(e) as being anticipated by U.S. Patent No. 7,014,737 to Harutyunyan ("Harutyunyan ") or in the alternative obvious under 35 U.S.C. §103(a) over Harutyunyan.

B. Whether Claims 8-9, 11-15 and 18 are unpatentable under 35 U.S.C. §102(e) as being anticipated by U.S. Patent No. 7,014,737 to Harutyunyan ("Harutyunyan ") or in the alternative obvious under 35 U.S.C. §103(a) over Harutyunyan.

C. Whether Claims 19, 21-24, and 27 are unpatentable under 35 U.S.C. §102(e) as being anticipated by U.S. Patent No. 7,014,737 to Harutyunyan ("Harutyunyan ") or in the alternative obvious under 35 U.S.C. §103(a) over Harutyunyan.

D. Whether Claims 1 and 7 are unpatentable under 35 U.S.C. §102(b) as being anticipated by WO 01/75903 to ('903) or in the alternative obvious under 35 U.S.C. §103(a) over '903.

E. Whether Claims 8-9, 11-12, and 18 are unpatentable under 35 U.S.C. §102(b) as being anticipated by WO 01/75903 to ('903) or in the alternative obvious under 35 U.S.C. §103(a) over '903.

F. Whether Claims 1 and 7 are unpatentable under 35 U.S.C. §102(b) as being anticipated by KO 2002-0046342 to ('342) or in the alternative obvious under 35 U.S.C. §103(a) over '342.

G. Whether Claims 8, 11-12, and 18 are unpatentable under 35 U.S.C. §102(b) as being anticipated by KO 2002-0046342 to ('342) or in the alternative obvious under 35 U.S.C. §103(a) over '342.

H. Whether Claims 5 and 6 are unpatentable under 35 U.S.C. §103(a) as being obvious over U.S. Patent No. 7,014,737 to Harutyunyan ("Harutyunyan ") in view of Fliflet *et al.* "Application of Microwave Heating to Ceramic Processing: Design and Initial Operation of a 2.45-GHz Single-Mode Furnace," IEEE Transactions on Plasma Science, Vol. 24, No. 3, June 1996, pp. 1041-1049 ("Fliflet").

I. Whether Claim 10 is unpatentable under 35 U.S.C. §103(a) as being obvious over U.S. Patent No. 7,014,737 to Harutyunyan ("Harutyunyan ") in view of Holtzinger *et al.* "Sidewall Functionalization of Carbon Nanotubes," Angew. Chem. Int. Ed. Engl., 2001, Vol. 40, No. 21, pp. 4002-4005 ("Holtzinger").

J. Whether Claims 16 and 17 are unpatentable under 35 U.S.C. §103(a) as being obvious over U.S. Patent No. 7,014,737 to Harutyunyan ("Harutyunyan ") in view of Fliflet *et al.* "Application of Microwave Heating to Ceramic Processing: Design and Initial Operation of a 2.45-GHz Single-Mode Furnace," IEEE Transactions on Plasma Science, Vol. 24, No. 3, June 1996, pp. 1041-1049 ("Fliflet").

K. Whether Claim 20 is unpatentable under 35 U.S.C. §103(a) as being obvious over U.S. Patent No. 7,014,737 to Harutyunyan ("Harutyunyan ") in view of Holtzinger *et al.* "Sidewall Functionalization of Carbon Nanotubes," Angew. Chem. Int. Ed. Engl., 2001, Vol. 40, No. 21, pp. 4002-4005 ("Holtzinger").

L. Whether Claims 25 and 26 are unpatentable under 35 U.S.C. §103(a) as being obvious over U.S. Patent No. 7,014,737 to Harutyunyan ("Harutyunyan ") in view of Fliflet *et al.* "Application of Microwave Heating to Ceramic Processing: Design and Initial Operation of a 2.45-GHz Single-Mode Furnace," IEEE Transactions on Plasma Science, Vol. 24, No. 3, June 1996, pp. 1041-1049 ("Fliflet").

VII. ARGUMENT

A. Claims 1-4 and 7 Are Not Properly Rejected Under 35 U.S.C. §102(e)/103(a): Harutyunyan

1. Claim 1
102(e)

An anticipation rejection of a claim under 35 U.S.C. §102(e) requires identity of invention; each and every feature of the claim must be identified by the Examiner, either explicitly or inherently, in a single prior art reference. Further, to establish inherency, extrinsic evidence must make clear that the missing descriptive matter is necessarily present in the device or system described in the reference, and that it would be so recognized by persons of ordinary skill in the art. *In re Robertson*, 169 F.3d 743, 49 USPQ2d 1949 (Fed. Cir. 1999). Inherency may not be established by probabilities or possibilities; the mere fact that a certain thing may result from a given set of circumstances is not sufficient to establish inherency. *Scaltech, Inc. v. Retech/ Tetra L.L.C.*, 156 F.3d 1193, 51 USPQ.2d 1055 (Fed. Cir. 1999).

Claim 1 is a method for crosslinking carbon nanotubes. The claim has the limitations of (1) providing the nanotubes, (2) irradiating the carbon nanotubes with microwaves, and (3) yielding a plurality of crosslinked nanotubes as a result of irradiating of the carbon nanotubes.

(1) The method of Harutyunyan targets radiation to the metal impurities found in carbon

nanotubes, leading to localized heating of the metal impurity and thence to the purification of carbon nanotubes.

(2) The Applicant's method, targets the radiation to the carbon nanotubes, resulting in crosslinking of the nanotubes.

The details of these different methods are explained further below.

On page 4, paragraph 3 of the Advisory Action the Examiner states that every feature of claim 1 has been identified in Harutyunyan referencing the Office Action of 3/14/07 at pages 5-6. The Examiner has further stated that Harutyunyan teaches a method of crosslinking carbon nanotubes. Office Action of 3/14/07, I at page 5, maintained Advisory Action of 5/24/07, I at page 3. Applicants traverse these assertions as detailed below.

First, Applicant objects to the characterization of Harutyunyan as teaching crosslinking. Harutyunyan teaches the purification of carbon nanotubes. Indeed, crosslinking of carbon nanotubes would be antithetical to the purification Harutyunyan seeks. Harutyunyan makes no mention at all of crosslinking, nor is it inherent as further explained below.

Harutyunyan achieves the purification of (1) nanotubes by (2) localized heating of the residual metal particle catalyst (which may be encased in carbon shells) with microwave radiation. For example, see the Abstract, col. 3, lines 22-25, and col. 4, lines 28-33. Indeed, Harutyunyan extols the virtue of using metal catalysts that have different physical properties from the carbon nanotubes to enable localized heating of the encased metal residue. See column 5, line 55-67 through column 6, lines 1-3. Again in column 6, lines 21-25, Harutyunyan states that the purification step "preferably includes a step of selectively inducing localized heating in the impurities,..." Thus, Harutyunyan discloses selectivity in absorption of the microwave radiation by the metal impurities and not the carbon nanotubes. Harutyunyan has not (1) supplied microwave radiation to the carbon nanotubes (the radiation is supplied to the metal impurities). For this reason Harutyunyan is not in possession of (2) a plurality of crosslinked nanotubes. To be clear, irradiation of a substrate without an interaction between the radiation and the substrate is operationally meaningless. Harutyunyan makes it very clear that the substrate interacting with the microwave radiation is the metal impurity, and not the carbon

nanotube. This is just one reason that the phrase "to yield a plurality of crosslinked carbon nanotubes," can not be ignored as a claim limitation. It modifies the irradiating step in that it clarifies that the carbon nanotubes have interacted with the microwave radiation, and have subsequently reacted as a result of this interaction. Because the carbon nanotubes have not been irradiated by Harutyunyan, one can not establish identity in the processes and thereby impose an argument of inherency.

In the Advisory Action at page 5, the Examiner argues that merely "exposing the crude reaction product comprising carbon nanotubes and the residual catalyst to microwave radiation," reads on Claim 1. The Examiner states that "to yield a plurality of crosslink [sic] carbon nanotubes" is the result of the performing the step and does not contribute to the operation of irradiating. Applicant traverses this line of reasoning. In order to successfully crosslink the carbon nanotubes one must necessarily target the microwave radiation such that the carbon nanotubes absorb the radiation. Harutyunyan does not target the carbon nanotubes. The Examiner can not disregard this operational limitation. It is descriptive of the interaction of the radiation with the carbon nanotubes.

Therefore Harutyunyan does not teach the technical features necessary for achieving Applicant's results. Of the stated limitations, Harutyunyan only provides carbon nanotubes. Therefore Claim 1 is patentable over Harutyunyan. Thus, withdrawal of this rejection is respectfully requested.

103(a)

Before a claim may be rejected under section 103, the examiner must establish a *prima facie* case of obviousness. See MANUAL OF PATENT EXAMINING PROCEDURE § 2142. A *prima facie* case consists of three elements. "There must be some suggestion or motivation . . . to modify the reference or combine the reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations."

In response to the Examiner's obviousness rejection of Claim 1, Harutyunyan's teachings are directed to the purification of carbon nanotubes. Modification of the Harutyunyan's procedure to crosslink carbon nanotubes would teach away from this very purpose. The required

modification would be to supply microwave radiation to the carbon nanotube rather than the metal impurity. By doing so Harutyunyan would no longer be able to obtain purified nanotubes; *i.e.* he would be destroying them by crosslinking them. The proposed modification cannot change the principle of operation of the prior art being modified, nor can the proposed modification render the prior art unsatisfactory for its intended purpose. *See* M.P.E.P. 2143.01, *see also In re Ratti*, 270 F.2d 810, 123 U.S.P.Q. 349 (CCPA 1959) and *In re Gordon*, 733 F.2d 900, 221 U.S.P.Q. 1125 (Fed. Cir. 1984), respectively.

In response to Applicant's teaching away argument, in the Advisory Action at page 6, the Examiner states yet again that "to yield a plurality of crosslink [sic] carbon nanotubes," does not make the method claim novel. For reasons stated above, the crosslinking of carbon nanotubes does help define how the irradiation is carried out, and the method of Harutyunyan is not carried out in the same manner. Carrying out the irradiation as taught by Harutyunyan teaches away from crosslinking. Applicant reiterates, carrying out the irradiation in the manner of Harutyunyan is useful for purifying carbon nanotubes. Crosslinking nanotubes, by Applicant's procedure, destroys the very operational purpose of Harutyunyan, to isolate purified carbon nanotubes. The proposed modification cannot change the principle of operation of the prior art being modified.

In the Advisory Action at page 7, second paragraph, the Examiner states that "Applicants have a different reason for, or advantage resulting from doing what the prior art relied upon has suggested,...". In response, no guidance is given to the reader of Harutyunyan that would suggest any modifications or motivation to change the procedure to crosslink nanotubes, by supplying the carbon nanotubes with microwave radiation. Harutyunyan's desire to purify nanotubes teaches away from modifying his procedure as stated above. The Examiner continues to overlook the limitation of crosslinking of the irradiated carbon nanotubes. This is descriptive of how the microwave radiation is actively interacting with the carbon nanotubes; *i.e.* irradiation is delivered to the carbon nanotubes, not the metal impurities as taught by Harutyunyan.

Applicant traverses the statement made by the Examiner that the Applicant is only defining the subject matter in terms of the results to be achieved...without providing the technical features necessary for achieving the result. (Office Action 3/14/07, page 6 last paragraph, *see also* Advisory Action at page 8, paragraphs 1 and 2) The desired result is

crosslinked carbon nanotubes. This is achieved by 1) providing carbon nanotubes and 2) irradiating the **carbon nanotubes** with microwave radiation as stated clearly in claim 1. Supplying the **carbon nanotubes** with microwave radiation is the technical feature that solves the problem of how to crosslink the carbon nanotubes. Harutyunyan **does not** irradiate the carbon nanotubes. Harutyunyan irradiates the **metal catalyst impurities**.

Harutyunyan fails to teach all the elements of the present invention. As explained above, the only limitation given by Harutyunyan is providing nanotubes. On page 7, paragraph 3, the Examiner states that there is no requirement to teach all the limitations to establish a *prima facie* case of obviousness. In response, the teaching of all the claim limitations by modification of and/or combining of references is one prong of establishing *prima facie* obviousness. A *prima facie* case consists of three elements. "There must be some suggestion or motivation . . . to modify the reference or combine the reference teachings. Second, there must be a reasonable expectation of success. **Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.**"

The Examiner has not established a *prima facie* case of obviousness because (1) there is no teaching or suggestion to modify Harutyunyan to crosslink nanotubes; (2) Harutyunyan teaches away from crosslinking nanotubes; and (3) Harutyunyan does not teach irradiating carbon nanotubes, nor obtaining crosslinked nanotubes. Harutyunyan only provides carbon nanotubes and does not provide the technical features necessary for achieving the Applicant's results.

Accordingly, Claim 1 is not obvious and is patentable over Harutyunyan. Applicants request withdrawal of this rejection.

2. Claim 2

102(e)/103(a)

Claim 2 incorporates all the limitations of Claim 1 and is therefore, patentable for at least the same reasons. Applicants request withdrawal of this rejection.

3. Claim 3

102(e)/103(a)

Claim 3 incorporates all the limitations of Claim 1, and is therefore, patentable for at least

the same reasons. Applicants request withdrawal of this rejection.

4. Claim 4

102(e)/103(a)

Claim 4 incorporates all the limitations of Claim 1, and is therefore, patentable for at least the same reasons. Applicant's request withdrawal of this rejection.

5. Claim 7

102(e)/103(a)

Claim 7 recites that the plurality of crosslinked carbon nanotubes comprises at least one junction formed via a rearrangement of carbon atoms. In addition to incorporating all the reasons stated above in Claim 1, nowhere does Harutyunyan disclose, teach, or suggest crosslinking that includes a junction formed via rearrangement of carbon atoms. The Examiner has failed to cite where Harutyunyan discloses or suggests this. This is because Harutyunyan does not teach any kind of crosslinking. Harutyunyan teaches irradiating the metal impurities rather than irradiating the carbon nanotubes. Applicants request withdrawal of this rejection.

B. Claims 8-9, 11-15 and 18 Are Not Properly Rejected Under 35 U.S.C. §102(e)/103(a): Harutyunyan

1. Claim 8

The Examiner has maintained rejections of Claim 8 for the similar reasons cited for maintaining the rejections of Claim 1. (Advisory Action at page 8-9 last paragraph)

102(e)

Claim 8 is a method for crosslinking nanotubes. The claim has the limitations of (1) providing the nanotubes, (2) irradiating the nanotubes with microwaves, (3) yielding a plurality of crosslinked nanotubes as a result of irradiating of the carbon nanotubes; and (4) crosslinking is generated between the sidewalls of adjacent carbon nanotubes.

In addition to the arguments presented above in Claim 1, the Examiner further has not pointed to a passage in Harutyunyan that discloses crosslinking between the sidewalls of adjacent carbon nanotubes, as stated in Claim 8. Again, this is because Harutyunyan irradiates the metal impurities not the carbon nanotubes. Therefore, there is no expectation that such a limitation would be inherent in Harutyunyan because the carbon nanotubes have not been

irradiated by Harutyunyan. Applicants irradiate the carbon nanotubes in order to obtain crosslinking. Harutyunyan does not irradiate the carbon nanotubes.

Applicants request withdrawal of this rejection.

103(a)

The same arguments presented above for Claim 1 hold for Claim 8. Additionally, crosslinking between the sidewalls of adjacent carbon nanotubes further teaches away from the very purpose of Harutyunyan, i.e. to purify carbon nanotubes. Crosslinking would destroy Harutyunyan's purpose of operation.

Applicants request withdrawal of this rejection.

2. Claim 9

102(e)/103(a)

Claim 9 incorporates all the limitations of Claim 8 and is therefore, patentable for at least the same reasons. Applicants request withdrawal of this rejection.

3. Claim 11

102(e)/103(a)

Claim 11 recites crosslinking comprising the formation of covalent bonds. In addition to incorporating all the reasons stated above in Claim 8, nowhere does Harutyunyan disclose, teach, or suggest the formation of covalent bonds between carbon nanotubes. The Examiner has failed to cite where Harutyunyan discloses or suggests this. This is because Harutyunyan does not teach any kind of covalent bond formation. Harutyunyan teaches irradiating the metal impurities rather than irradiating the carbon nanotubes. Because the carbon nanotubes have not been irradiated by Harutyunyan, one can not establish identity in the processes and thereby impose an argument of inherency. Applicants request withdrawal of this rejection.

4. Claim 12

102(e)/103(a)

Claim 12 recites crosslinking comprising the formation of covalent bonds that are carbon-carbon bonds. In addition to incorporating all the reasons stated above in Claim 8, nowhere does Harutyunyan disclose, teach, or suggest the formation of covalent carbon-carbon bonds between carbon nanotubes. The Examiner has failed to cite where Harutyunyan discloses or suggests this.

This is because Harutyunyan does not teach any kind of covalent bond formation. Harutyunyan teaches irradiating the metal impurities rather than irradiating the carbon nanotubes. Because the carbon nanotubes have not been irradiated by Harutyunyan, one can not establish identity in the processes and thereby impose an argument of inherency. Applicants request withdrawal of this rejection.

5. Claim 13

102(e)/103(a)

Claim 13 incorporates all the limitations of Claim 8 and is therefore patentable for at least the same reasons. Applicants request withdrawal of this rejection.

6. Claim 14

102(e)/103(a)

Claim 14 incorporates all the limitations of Claim 8 and is therefore patentable for at least the same reasons. Applicants request withdrawal of this rejection.

7. Claim 15

102(e)/103(a)

Claim 15 incorporates all the limitations of Claim 8 and is therefore patentable for at least the same reasons. Applicants request withdrawal of this rejection.

8. Claim 18

102(e)/103(a)

Claim 18 recites that the plurality of crosslinked carbon nanotubes comprises at least one junction formed via a rearrangement of carbon atoms. In addition to incorporating all the reasons stated above in Claim 8, nowhere does Harutyunyan disclose, teach, or suggest crosslinking that includes a junction formed via rearrangement of carbon atoms. The Examiner has failed to cite where Harutyunyan discloses or suggests this. This is because Harutyunyan does not teach any kind of crosslinking. Harutyunyan teaches irradiating the metal impurities rather than irradiating the carbon nanotubes. Because the carbon nanotubes have not been irradiated by Harutyunyan, one can not establish identity in the processes and thereby impose an argument of inherency. Applicants request withdrawal of this rejection.

C. Claims 19, 21-24, and 27 Are Not Properly Rejected Under 35 U.S.C.

§102(e)/103(a): Harutyunyan

1. Claim 19

The Examiner has maintained rejections of Claim 19 for the same reasons cited for in the Office Action of 3/14/07. (Advisory Action at page 9 last paragraph)

102(e)

Claim 19 is a method for crosslinking nanotubes. The claim has the limitations of (1) providing the nanotubes, (2) irradiating the nanotubes with microwaves, (3) yielding a plurality of crosslinked nanotubes as a result of irradiating of the carbon nanotubes; (4) crosslinking is generated between the sidewalls of adjacent carbon nanotubes; and (5) the irradiation is carried out in an inert environment.

The arguments presented above in Claims 1 and 8 apply to Claim 19.

Applicants request withdrawal of this rejection.

103(a)

The same arguments presented above for Claims 1 and 8 hold for Claim 19. Applicants request withdrawal of this rejection.

2. Claim 21

102(e)/103(a)

Claim 21 recites crosslinking comprising the formation of covalent bonds. In addition to incorporating all the reasons stated above in Claims 1, 8, and 19 nowhere does Harutyunyan disclose, teach, or suggest the formation of covalent bonds between carbon nanotubes. The Examiner has failed to cite where Harutyunyan discloses or suggests this. This is because Harutyunyan does not teach any kind of covalent bond formation. Harutyunyan teaches irradiating the metal impurities rather than irradiating the carbon nanotubes. Because the carbon nanotubes have not been irradiated by Harutyunyan, one can not establish identity in the processes and thereby impose an argument of inherency. Applicants request withdrawal of this rejection.

3. Claim 22

102(e)/103(a)

Claim 22 recites crosslinking comprising the formation of covalent bonds that are carbon-

carbon bonds. In addition to incorporating all the reasons stated above in Claims 1, 8, and 19 nowhere does Harutyunyan disclose, teach, or suggest the formation of covalent carbon-carbon bonds between carbon nanotubes. The Examiner has failed to cite where Harutyunyan discloses or suggests this. This is because Harutyunyan does not teach any kind of covalent bond formation. Harutyunyan teaches irradiating the metal impurities rather than irradiating the carbon nanotubes. Because the carbon nanotubes have not been irradiated by Harutyunyan, one can not establish identity in the processes and thereby impose an argument of inherency. Applicants request withdrawal of this rejection.

4. Claim 23

102(e)/103(a)

Claim 23 incorporates all the limitations of Claim 19 and is therefore patentable for at least the same reasons. Applicants request withdrawal of this rejection.

5. Claim 24

102(e)/103(a)

Claim 24 incorporates all the limitations of Claim 19 and is therefore patentable for at least the same reasons. Applicants request withdrawal of this rejection.

6. Claim 27

102(e)/103(a)

Claim 27 recites that the plurality of crosslinked carbon nanotubes comprises at least one junction formed via a rearrangement of carbon atoms. In addition to incorporating all the reasons stated above in Claims 1 and 8, as well as depending from patentably distinct Claim 19, nowhere does Harutyunyan disclose, teach, or suggest crosslinking that includes a junction formed via rearrangement of carbon atoms. The Examiner has failed to cite where Harutyunyan discloses or suggests this. This is because Harutyunyan does not teach any kind of crosslinking. Harutyunyan teaches irradiating the metal impurities rather than irradiating the carbon nanotubes. Because the carbon nanotubes have not been irradiated by Harutyunyan, one can not establish identity in the processes and thereby impose an argument of inherency. Applicants request withdrawal of this rejection.

D. Claims 1 and 7 Are Not Properly Rejected Under 35 U.S.C. §102(b)/103(a): WO 01/75903 ('903).

1. Claim 1

102(b)

Claim 1 recites a method for crosslinking carbon nanotubes comprising the steps of (1) providing carbon nanotubes and (2) irradiating said nanotubes with microwave radiation to crosslink the carbon nanotubes.

The Examiner has stated that the '903 publication teaches a method of crosslinking carbon nanotubes (CNTs). Applicant respectfully disagrees with this characterization of the reference and points out that '903 application does not teach crosslinking CNTs, but rather teaches conducting materials containing both nanostructures and a charge-transfer agent (preferably a metal matrix material) that can transfer charge between itself and the nanostructure. Furthermore, the charge transfer agent is adapted to shift the Fermi level of the nanostructure to attain enhanced conductivity. See claim 1 of the '903 patent, for example. That is, the '903 patent provides nanostructures within a charge transfer agent (matrix) as part of a conducting material. This construct is not subjected to microwave radiation.

The '903 application does contain a short theoretical discussion about the interaction of nanostructures with electromagnetic radiation as part of a discussion on band gap energy:

"By using electromagnetic radiation, such as microwaves or light to irradiate nanostructures, excited electrons are produced...." ('903 at page 9 lines 19-20)

The term nanostructure covers an infinite array of possible chemical compositions and constructs. Furthermore, all that is disclosed is that a nanostructure can have its valence band electrons excited by interaction with electromagnetic radiation. The problem with this logic is that this is true of all molecules! The disclosure calls out microwave radiation as an example of electromagnetic radiation without disclosing what types of nanostructures can absorb microwave radiation, and not all nanostructures will absorb microwave radiation. There is no disclosure that carbon nanotubes can absorb microwave radiation, in particular. There is no specific reference even made to carbon nanotubes in this theoretical discussion. Furthermore, there is no specific disclosure of irradiating carbon nanotubes with microwave radiation to crosslink them. Thus, the '903 patent fails to disclose any of the claim limitations as presented in Claim 1. There is no

disclosure specifically (1) providing carbon nanotubes in this theoretical treatise. There is no disclosure specifically stating (2) irradiation of carbon nanotubes with microwaves as stated in Claim 1. Finally, there is no discussion of (3) yielding a plurality of crosslinked carbon nanotubes. What is disclosed in the theoretical discussion in the '903 application are general principles related to band gap energies and the excitation of electrons in matter when subjected to electromagnetic radiation. Applicant request withdrawal of this rejection.

103(a)

Statements of the general scientific principles of excitation of valence band electrons by the interaction of electromagnetic radiation with nanostructures fails to teach any of the claim limitations of claim 1. Knowing these general principles does not suggest or guide the reader of the '903 patent to put together all the claim limitations as recited in Claim 1. There are an infinite number of potential nanostructures that could be subjected to *any* electromagnetic radiation and there is no guidance in the '903 patent to construct the limitations as presented in Claim 1. In fact, there is no teaching that electron excitation generally leads to crosslinking in molecules. It is fair to say that excited state chemistry of molecules can be quite complex and unpredictable with many more processes other than crosslinking that may occur for a given molecule. Such processes may include relaxation back to the ground state (via intersystem crossing--typically in photoexcitation), fragmentation reactions, atom abstraction processes, charge transfer, and simple dimerization, to name a few. Even assuming *arguendo* that one chooses carbon nanotubes and microwave radiation, the '903 patent only teaches that electrons will be excited. There would be no expectation of success in crosslinking the carbon nanotubes based solely on the principles of band gap energy discussed in the '903 patent. The Examiner has made broad conclusory statements that do not support a *prima facie* case of obviousness. Furthermore, the subject matter as a whole of the '903 patent has nothing to do with crosslinking carbon nanotubes. The '903 patent is concerned with enhanced conductivity of nanotubes in charge transfer matrices. In this pursuit, irradiation with microwaves is not even a part of the discussion. Indeed, in the preferred embodiment the charge transfer material is a metal. Anyone that has left a metal fork in a microwave oven knows how badly that can turn out. Applicants request withdrawal of this rejection.

2. Claim 7

102(b)/103(a)

Claim 7 recites that the plurality of crosslinked carbon nanotubes comprise at least one junction formed via a rearrangement of carbon atoms. In addition to incorporating all the reasons stated above in Claim 1, nowhere does the '903 application disclose, teach, or suggest crosslinking that includes a junction formed via rearrangement of carbon atoms. The Examiner has failed to cite where the '903 application discloses or suggests this. This is because the '903 does not teach any kind of crosslinking. The '903 application merely states that nanostructures may have their valence band electrons excited by absorption of electromagnetic radiation. As stated above this is true for any molecule. Applicants request withdrawal of this rejection.

E. Claims 8-9, 11-12, and 18 Are Not Properly Rejected Under 35 U.S.C. §102(b)/103(a): WO 01/75903 ('903).

1. Claim 8

102(b)

Claim 8 is a method for crosslinking nanotubes. The claim has the limitations of (1) providing the nanotubes, (2) irradiating the nanotubes with microwaves, (3) yielding a plurality of crosslinked nanotubes as a result of irradiating of the carbon nanotubes; and (4) crosslinking is generated between the sidewalls of adjacent carbon nanotubes.

In addition to the arguments presented above in Claim 1, the Examiner further has not pointed to a passage in the '903 application that discloses crosslinking between the sidewalls of adjacent carbon nanotubes, as stated in Claim 8. Indeed, none of the claim limitations appear to be present in the '903 application with regard to Claim 8 as in Claim 1. Applicants request withdrawal of this rejection.

103(a)

The same arguments presented above for Claim 1 hold for Claim 8 regarding obviousness. Further, nothing teaches or suggests the details of the chemistry of carbon nanotubes when irradiated with microwave radiation. Applicants request withdrawal of this rejection.

2. Claim 9

102(e)/103(a)

Claim 9 incorporates all the patentable limitations of Claim 8. Claim 9 further recites

that the carbon nanotubes are single-wall carbon nanotubes. The '903 patent merely teaches that valence band electrons in nanostructures may be excited by electromagnetic radiation. Nowhere is there a suggestion that one ought to pick out any kind of nanotube from the infinite possibilities of nanostructures available, let alone a single-wall carbon nanotube. Applicants request withdrawal of this rejection.

3. Claim 11

102(e)/103(a)

Claim 11 recites crosslinking comprising the formation of covalent bonds. In addition to incorporating all the reasons stated above in Claim 8, nowhere does the '903 application disclose, teach, or suggest the formation of covalent bonds between carbon nanotubes. The Examiner has failed to cite where the '903 application discloses or suggests this. This is because the '903 application does not teach any kind of covalent bond formation. The '903 patent merely teaches that valence band electrons in nanostructures may be excited by electromagnetic radiation. Applicants request withdrawal of this rejection.

4. Claim 12

102(e)/103(a)

Claim 12 recites crosslinking comprising the formation of covalent bonds that are carbon-carbon bonds. In addition to incorporating all the reasons stated above in Claim 8, nowhere does the '903 application disclose, teach, or suggest the formation of covalent carbon-carbon bonds between carbon nanotubes. The Examiner has failed to cite where the '903 application discloses or suggests this. This is because the '903 application does not teach any kind of covalent bond formation. The '903 patent merely teaches that valence band electrons in nanostructures may be excited by electromagnetic radiation. Applicants request withdrawal of this rejection.

8. Claim 18

102(e)/103(a)

Claim 18 recites that the plurality of crosslinked carbon nanotubes comprises at least one junction formed via a rearrangement of carbon atoms. In addition to incorporating all the reasons stated above in Claim 8, nowhere does the '903 application disclose, teach, or suggest crosslinking that includes a junction formed via rearrangement of carbon atoms. The Examiner

has failed to cite where the '903 application discloses or suggests this. This is because the '903 application does not teach any kind of crosslinking. The '903 patent merely teaches that valence band electrons in nanostructures may be excited by electromagnetic radiation. Applicants request withdrawal of this rejection.

F. Claims 1 and 7 Are Not Properly Rejected Under 35 U.S.C. §102(b)/103(a):KR 2002-0046342 ('342).

1. Claim 1

102(b)

Claim 1 recites a method for crosslinking carbon nanotubes comprising the steps of (1) providing carbon nanotubes and (2) irradiating said nanotubes with microwave radiation to crosslink the carbon nanotubes.

The Examiner has not presented a *prima facie* case of anticipation because KR '342 does not disclose crosslinking, either expressly or inherently. As clearly stated in the description of KR '342, the helical coiled nanotube is irradiated so that heat is generated at the local section of the body. This is not crosslinking. The Examiner has not provided a line of reason for why crosslinking should be inherent.

The Examiner has stated that KR '342 teaches a method of crosslinking carbon nanotubes (CNTs). Applicant respectfully disagrees and points out that KR '342 does not disclose crosslinking CNTs, but rather discloses using an encapsulated helical coiled carbon nanotube to provide local heating to cancerous tissue.

Applicant pointed out in response to the Office Action of 3/14/07 that the isolated carbon nanotubes of KR '342 can not crosslink due to their isolation as solitary carbon nanotubes. The Examiner in the Advisory Action (page 17, paragraph 2) points to Figure 1 on page 8 to show the presence of multiple helical coiled nanotubes. This Figure does not constitute proof that once delivered to the tissue, the coiled helical tubes will crosslink. Nor is there reason to suggest that because the encapsulation may be broken in KR '342, that this alone will allow crosslinking to occur. Indeed the ability to crosslink may depend on an enormous number of factors not present in the conditions of the irradiation of KR '342. The presence of the solvent, water, alone can and likely will change the course of the irradiation. Crosslinking at the very least in the KR '342

patent is going to require diffusion of the nanotubes through the aqueous media to come into a range where they can contact each other sufficiently to form covalent crosslinks as described in the present application.

Even assuming *arguendo* that carbon nanotubes may diffuse and come into close range of each other for reaction, the Examiner has not given a good reason why coiled nanotubes should inherently react in a crosslinking manner.

Inherency may not be established by probabilities or possibilities; the mere fact that a certain thing may result from a given set of circumstances is not sufficient to establish inherency. *Scaltech, Inc. v. Retech/ Tetra L.L.C.*, 156 F.3d 1193, 51 USPQ.2d 1055 (Fed. Cir. 1999). In fact a helical coiled carbon nanotube interacts differently with **any** type of electromagnetic radiation, including microwave radiation, compared to non-coiled carbon nanotubes of the present application.

To illustrate the differences between typical carbon nanotubes of the present application and the coiled helical tubes of KR '342, there are several relevant passages to consider. In the present application at paragraph [0026] on page 8, Applicant discloses that the heat release upon exposure of carbon nanotubes (of the present application) to microwave radiation can generate temperatures as high as 2000 °C.

These kinds of damaging temperatures are not envisioned by KR '342 which characterizes the prior art heating methods as follows:

"The shortcoming of this local heat therapy is in some cases the development of burn symptoms because an abnormally high temperature is produced." (Page 3, paragraph 2)

A person of reason is likely to find temperatures as high as 2000 °C incompatible with the therapy treatments envisioned in KR '342. Submitted along with this appeal brief is a supporting article by Dickson *et al.* that shows RF heating of tumors in rabbits. The temperatures necessary to show tumor regression were a mere 47 °C.

Further demonstration of different interactions of electromagnetic radiation with helical coils of carbon nanotubes are found in the KR '342 description:

"In addition, when the electromagnetic wave with a wavelength longer than the length of the microscopic coil of the carbon nanotube enters, the

magnetic field inside the coil changes and the microscopic coil of the carbon nanotube generates induced electromotive force in the opposite direction of the change [sic] and current flow is produced. When this phenomenon is interpreted by the magnetic field, the changes in the flux of the magnetic field within the microscopic coil produce a current flow which is opposite to the flux of the magnetic field and thereby form a magnetic flux which is the opposite to the flux of the magnetic field.

Such a mechanism is certainly accompanied by heat generation."

This passage explains a phenomenon that exists only in coiled carbon nanotubes, i.e. the same effect as running current through a coiled wire. Clearly, the interaction of electromagnetic radiation with the coiled carbon nanotube structure is different from a typical non-coiled structure. The burden of showing that these coiled carbon nanotubes can crosslink in the manner claimed by Applicants has not been met. The evidence presented here shows that there is a substantial difference in the way the two entities interact with electromagnetic radiation. Applicant therefore suggests that showing inherency is highly unlikely, especially in this unpredictable art.

The Examiner has not met the burden of demonstrating *prima facie* anticipation of the claims of the present application and thus, Claim 1 is patentable over KR '342. Thus, withdrawal of this rejection is respectfully requested.

103(a)

In response to the Examiner's obviousness rejection of Claim 1, KR '342 teaches local heating in cancerous tissues using irradiation of helical carbon nanotubes delivered to the tissue. For the reasons given above, it is unlikely that such coiled carbon nanotubes will inherently crosslink. In fact, purposely crosslinking the coiled nanotubes of KR '342 renders them inoperable for their intended function. This is because the crosslinking would alter the ability of the coiled nanotubes to continually provide heat by altering their absorption and emission properties. A proposed modification cannot change the principle of operation of the prior art being modified, nor can the proposed modification render the prior art unsatisfactory for its intended purpose. See M.P.E.P. 2143.01, *see also In re Ratti*, 270 F.2d 810, 123 U.S.P.Q. 349 (CCPA 1959) and *In re Gordon*, 733 F.2d 900, 221 U.S.P.Q. 1125 (Fed. Cir. 1984), respectively.

KR '342 (1) fails to teach all the elements of the present invention as described above and (2) crosslinking render KR '342 inoperable for its intended purpose. Thus, there is a teaching

away from crosslinking the coiled nanotubes. Not only is crosslinking not inherently a part of the KR '342 process it would be undesirable. For these reasons, the Examiner has not established a *prima facie* case of obviousness. Thus, withdrawal of this rejection is respectfully requested.

2. Claim 7

102(b)/103(a)

Claim 7 recites that the plurality of crosslinked carbon nanotubes comprise at least one junction formed via a rearrangement of carbon atoms. In addition to incorporating all the reasons stated above in Claim 1, nowhere does the KR '342 disclose, teach, or suggest crosslinking that includes a junction formed via rearrangement of carbon atoms. The Examiner has failed to cite where KR '342 discloses or suggests this. This is because KR '342 does not teach any kind of crosslinking. KR '342 merely provides non-damaging heating of tissues by delivering encapsulated helical coiled carbon nanotubes and then providing some electromagnetic means of generating a current through the coil. Applicants request withdrawal of this rejection.

G. Claims 8, 11-12, And 18 Are Not Properly Rejected Under 35 U.S.C. §102(b)/103(a):KO 2002-0046342 ('342).

1. Claim 8

102(b)

Claim 8 is a method for crosslinking nanotubes. The claim has the limitations of (1) providing the nanotubes, (2) irradiating the nanotubes with microwaves, (3) yielding a plurality of crosslinked nanotubes as a result of irradiating of the carbon nanotubes; and (4) crosslinking is generated between the sidewalls of adjacent carbon nanotubes.

The Examiner has not presented a *prima facie* case of anticipation because KR '342 does not disclose **crosslinking**, either expressly or inherently. As clearly stated in the description of KR '342, the helical coiled nanotube is irradiated so that heat is generated at the local section of the body. This is not crosslinking. The Examiner has not provided a line of reason for why crosslinking should be inherent.

Furthermore, there is no disclosure of reactions between sidewalls of the coiled helical carbon nanotube structures, either expressly or inherently.

In addition to the applicability of the arguments presented above in Claim 1, the Examiner has not pointed to a passage in KR '342 that discloses crosslinking between the sidewalls of adjacent carbon nanotubes, as stated in Claim 8. Indeed is it not even clear that the manner in which the coiled nanotubes are delivered that they will be disposed in proximity to each other for reaction as described above for Claim 1. Applicants request withdrawal of this rejection.

103(a)

The same arguments presented above for Claim 1 hold for Claim 8 regarding obviousness. Applicants request withdrawal of this rejection.

3. Claim 11

102(e)/103(a)

Claim 11 recites crosslinking comprising the formation of covalent bonds. In addition to incorporating all the reasons stated above in Claim 8, nowhere does KR '342 disclose, teach, or suggest the formation of covalent bonds between carbon nanotubes. The Examiner has failed to cite where KR '342 discloses or suggests this. This is because KR '342 does not teach any kind of covalent bond formation between coiled carbon nanotubes. Applicants request withdrawal of this rejection.

4. Claim 12

102(e)/103(a)

Claim 12 recites crosslinking comprising the formation of covalent bonds that are carbon-carbon bonds. In addition to incorporating all the reasons stated above in Claim 8, nowhere does KR '342 disclose, teach, or suggest the formation of covalent carbon-carbon bonds between carbon nanotubes. The Examiner has failed to cite where KR '342 discloses or suggests this. This is because KR '342 does not teach any kind of covalent bond formation between coiled carbon nanotubes. Applicants request withdrawal of this rejection.

8. Claim 18

102(e)/103(a)

Claim 18 recites that the plurality of crosslinked carbon nanotubes comprises at least one junction formed via a rearrangement of carbon atoms. In addition to incorporating all the reasons stated above in Claim 8, nowhere does KR '342 disclose, teach, or suggest crosslinking that includes a junction formed via rearrangement of carbon atoms. The Examiner has failed to cite where KR '342 discloses or suggests this. This is because KR '342 does not teach any kind of crosslinking. Applicants request withdrawal of this rejection.

H. Claims 5 And 6 Are Not Properly Rejected Under 35 U.S.C. §103(a): No. Harutyunyan in view of Fliflet

Claims 5 and 6 state that the microwave radiation is generated by a magnetron with a power that ranges from about 1 W to about 10,000 W (Claim 5) or, more narrowly from about 10 W to about 1,000 W (Claim 6). All the reasons for the patentability of independent Claim 1 over Harutyunyan are incorporated herein and Claims 5 and 6 are patentable for at least those stated reasons. As specifically concerning Claims 5 and 6, the Examiner states that it would have been obvious replace the 1.5 kW/2.45 GHz power supply of the tuned TE103 single mode cavity with Fliflet's NRL 2.45 GHz microwave furnace. Absent the Applicant's disclosure there is absolutely no reason to make such a substitution. The Examiner must find the motivation to make such a change in the references cited. The Examiner has merely used the Applicant's disclosure as a road map for hindsight reconstruction to suggest changing the microwave source. The teaching or suggestion to make the claimed combination (or in this case replacement) and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 492–94 (Fed. Cir. 1991). Applicant requests withdrawal of this rejection.

I. Claim 10 Is Not Properly Rejected Under 35 U.S.C. §103(a): Harutyunyan in view of Holtzinger

Claim 10 recites that the carbon nanotubes are chemically functionalized prior to the step of irradiating. All the reasons for the patentability of independent Claim 8 over Harutyunyan are incorporated herein and Claim 10 is patentable for at least those stated reasons. Harutyunyan is concerned with purifying carbon nanotubes. He accomplishes this by irradiating the **metal impurities**. One could replace the unfunctionalized nanotubes of Harutyunyan with the

functionalized nanotubes of Holtzinger, but what is the motivation to make such a replacement? Harutyunyan is selectively irradiating the metal impurities rather than the nanotubes so the change from unfunctionalized tubes to functionalized tubes is inconsequential to the Harutyunyan process, as long as he maintains his selectivity. Claim 10 is directed to the crosslinking of functionalized carbon nanotubes by irradiating the nanotubes with microwave radiation. In view of the disparity between the teachings of Harutyunyan and the Applicant's crosslinking process, the switch to functionalized nanotubes is structurally relevant only with in the Applicant's process. Harutyunyan's teaching only concern purifying nanotubes by irradiating the metal impurities. Applicant requests withdrawal of this rejection.

J. Claims 16 And 17 Are Not Properly Rejected Under 35 U.S.C. §103(a): Harutyunyan in view of Fliflet

Claims 16 and 17 state that the microwave radiation is generated by a magnetron with a power that ranges from about 1 W to about 10,000 W (Claim 16) or, more narrowly from about 10 W to about 1,000 W (Claim 17). All the reasons for the patentability of independent Claim 8 over Harutyunyan are incorporated herein and Claims 16 and 17 are patentable for at least those stated reasons. As specifically concerning Claims 16 and 17, the Examiner states that it would have been obvious replace the 1.5 kW/2.45 GHz power supply of the tuned TE103 single mode cavity with Fliflet's NRL 2.45 GHz microwave furnace. Absent the Applicant's disclosure there is absolutely no reason to make such a substitution. The Examiner must find the motivation to make such a change in the references cited. The Examiner has merely used the Applicant's disclosure as a road map for hindsight reconstruction to suggest changing the microwave source. The teaching or suggestion to make the claimed combination (or in this case replacement) and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 492-94 (Fed. Cir. 1991). Applicant requests withdrawal of this rejection.

K. Claim 20 Is Not Properly Rejected Under 35 U.S.C. §103(a): Harutyunyan in view of Holtzinger

Claim 20 recites that the carbon nanotubes are chemically functionalized prior to the step of irradiating. All the reasons for the patentability of independent Claim 19 over Harutyunyan are incorporated herein and Claim 20 is patentable for at least those stated reasons. Harutyunyan is concerned with purifying carbon nanotubes.

He accomplishes this by irradiating the metal impurities. One could replace the unfunctionalized nanotubes of Harutyunyan with the functionalized nanotubes of Holtzinger, but what is the motivation to make such a replacement? Harutyunyan is selectively irradiating the metal impurities rather than the nanotubes so the change from unfunctionalized tubes to functionalized tubes is inconsequential to the Harutyunyan process, as long as he maintains his selectivity. Claim 10 is directed to the crosslinking of functionalized carbon nanotubes by irradiating the nanotubes with microwave radiation. In view of the disparity between the teachings of Harutyunyan and the Applicant's crosslinking process, this rejection is a *non sequitur*. Applicant requests withdrawal of this rejection.

L. Claims 25 and 26 Are Not Properly Rejected Under 35 U.S.C. §102(b)/103(a): Harutyunyan in view of Fliflet

Claims 25 and 26 state that the microwave radiation is generated by a magnetron with a power that ranges from about 1 W to about 10,000 W (Claim 25) or, more narrowly from about 10 W to about 1,000 W (Claim 26). All the reasons for the patentability of independent Claim 19 over Harutyunyan are incorporated herein and Claims 25 and 26 are patentable for at least those stated reasons. As specifically concerning Claims 25 and 26, the Examiner states that it would have been obvious replace the 1.5 kW/2.45 GHz power supply of the tuned TE103 single mode cavity with Fliflet's NRL 2.45 GHz microwave furnace. Absent the Applicant's disclosure there is absolutely no reason to make such a substitution. The Examiner must find the motivation to make such a change in the references cited. The Examiner has merely used the Applicant's disclosure as a road map for hindsight reconstruction to suggest changing the microwave source. The teaching or suggestion to make the claimed combination (or in this case replacement) and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 492–94 (Fed. Cir. 1991). Applicant requests withdrawal of this rejection.

CONCLUSION

For the reasons noted above, the rejections of Claims 1-27 are in error. Appellants respectfully request reversal of the rejections and allowance of claims 1-27.

Respectfully submitted,

WINSTEAD PC

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VIII. CLAIMS APPENDIX

1. A method of crosslinking carbon nanotubes comprising:
 - (1) providing carbon nanotubes; and
 - (2) irradiating said carbon nanotubes with microwaves to yield a plurality of crosslinked carbon nanotubes.
2. The method of claim 1, wherein the step of irradiating is carried out in an inert environment selected from the group consisting of ultra-high vacuum, high vacuum, inert gases, and combinations thereof.
3. The method of claim 1, wherein the microwave radiation comprises a frequency that ranges from about 0.01 GHz to about 100 GHz.
4. The method of claim 3, wherein the frequency ranges from about 1 GHz to about 18 GHz.
5. The method of claim 1, wherein the microwave radiation is generated by a magnetron with a power that ranges from about 1 W to about 10,000 W.
6. The method of claim 5, wherein the power ranges from about 10 W to about 1,000 W.
7. The method of claim 1, wherein the plurality of crosslinked carbon nanotubes comprises at least one junction formed via a rearrangement of carbon atoms.
8. A method of crosslinking carbon nanotubes comprising:
 - (1) providing carbon nanotubes; and
 - (2) irradiating said carbon nanotubes with microwaves to yield a plurality of crosslinked carbon nanotubes;
wherein crosslinking is generated between the sidewalls of adjacent carbon nanotubes.
9. The method of claim 8, wherein the carbon nanotubes are single-wall carbon nanotubes.
10. The method of claim 8, wherein the carbon nanotubes are chemically functionalized prior to the step of irradiating.
11. The method of claim 8, wherein the crosslinking comprises covalent bonds.
12. The method of claim 11, wherein the covalent bonds are carbon-carbon bonds.
13. The method of claim 8, wherein the step of irradiating is carried out in an inert environment selected from the group consisting of ultra-high vacuum, high vacuum, inert gases, and

combinations thereof.

14. The method of claim 8, wherein the microwave radiation comprises a frequency that ranges from about 0.01 GHz to about 100 GHz.
15. The method of claim 14, wherein the frequency ranges from about 1 GHz to about 18 GHz.
16. The method of claim 8, wherein the microwave radiation is generated by a magnetron with a power that ranges from about 1 W to about 10,000 W.
17. The method of claim 16, wherein the power ranges from about 10 W to about 1,000 W.
18. The method of claim 8, wherein the plurality of crosslinked carbon nanotubes comprises at least one junction formed via a rearrangement of carbon atoms.
19. A method of crosslinking single-wall carbon nanotubes comprising:
 - (1) providing single-wall carbon nanotubes; and
 - (2) irradiating said single-wall carbon nanotubes with microwaves to yield a plurality of crosslinked single-wall carbon nanotubes;

wherein crosslinking is generated between the sidewalls of adjacent single-wall carbon nanotubes; and

wherein the step of irradiating is carried out in an inert environment selected from the group consisting of ultra-high vacuum, high vacuum, inert gases, and combinations thereof.
20. The method of claim 19, wherein the single-wall carbon nanotubes are chemically functionalized prior to the step of irradiating.
21. The method of claim 19, wherein the crosslinking comprises covalent bonds.
22. The method of claim 21, wherein the covalent bonds are carbon-carbon bonds.
23. The method of claim 19, wherein the microwave radiation comprises a frequency that ranges from about 0.01 GHz to about 100 GHz.
24. The method of claim 23, wherein the frequency ranges from about 1 GHz to about 18 GHz.
25. The method of claim 19, wherein the microwave radiation is generated by a magnetron with a power that ranges from about 1 W to about 10,000 W.
26. The method of claim 25, wherein the power ranges from about 10 W to about 1,000 W.
27. The method of claim 19, wherein the plurality of crosslinked single-wall carbon nanotubes comprises at least one junction formed via a rearrangement of carbon atoms.

IX. EVIDENCE APPENDIX

No evidence was submitted pursuant to §§1.130, 1.131, or 1.132 of 37 C.F.R. Attached with this appeal is Exhibit A, which we cite herein upon appeal: Dickson et al. "Tumor Eradication in the Rabbit by Radiofrequency Heating," Cancer Res. 1977, 2162.

X. RELATED PROCEEDINGS APPENDIX

There are no related proceedings to the current proceedings.

EXHIBIT A

Tumor Eradication in the Rabbit by Radiofrequency Heating¹

J. A. Dickson, S. A. Shah, D. Waggott, and W. B. Whalley

Cancer Research Unit, University Department of Clinical Biochemistry, Royal Victoria Infirmary, Newcastle upon Tyne, England [J. A. D., S. A. S.], and Critical Systems Incorporated, Palo Alto, California 94302 [D. W., W. B. W.]

SUMMARY

A radiofrequency (RF) machine operating at the lower end of the short-wave medical diathermy range (13.56 MHz, 22-m wavelength) has been designed for heating tumors. The apparatus employs the electrostatic (condenser) field technique, whereby the tissue becomes part of the output circuit and is heated between two paddle electrodes applied to opposite sides of the tumor. Incorporation of tuned compensating coils in the paddle handles, a connector lead between the paddles, and a d.c. inverse feedback loop between the RF output and the crystal oscillator are special features of the circuitry developed to produce stable and readily controlled tumor heating.

Thermistor and thermocouple needle-type sensors were examined for tissue temperature measurement in the low-power RF fields. At power levels up to 6 watts, temperature readings obtained with thermistors or copper-constantan thermocouples, in association with analog recording systems, were unaffected by the RF field. At 6- to 12-watt output, only the thermocouple-galvanometer system remained unaffected, the thermistors reading consistently 1.0–1.5° high. Digital meters with thermocouples proved to be less stable recording systems in these RF fields.

With the machine, i.m. VX2 carcinomas (up to 22 ml in volume) in the hind limb of rabbits were heated at 47° for 30 min. A temperature differential of 2–3° was usually recorded between multiple sensors in large tumors. The temperature of skin and normal muscle within the RF field remained 3–4° below the minimal temperature in the tumor, and no marked elevation of body temperature occurred. Seven out of 10 VX2 carcinomas given a single treatment at 47° regressed completely, with cure of the host and without damage to normal tissues. At the time of heating, metastases were present in the regional and distant lymph nodes and in the lungs.

The presence of tumor in the muscle did not significantly alter the electrical resistance of the leg, and the results suggest that the selective heating of the VX2 carcinoma in the RF field may be conditioned by poor blood flow through the tumor compared with normal adjacent tissue.

INTRODUCTION

As the result of recent reports and ongoing investigations in numerous centers, the idea is rapidly gaining ground that

hyperthermia may have considerable potential as an antitumor modality (3, 13, 21, 36). There is good evidence that at 42° a metabolic Achilles heel may exist in many types of cancer cells, as indicated by a selective inhibition of respiration, glycolysis, and nucleic acid and protein synthesis (3, 13, 23, 24). Attention has also been directed to the potentiating role of hyperthermia in the destruction of malignant cells by radiotherapy (30, 33), drugs (1, 14), and cell-specific antiserum (17).

Fundamental information is becoming available on the factors governing tumor response to heat, such as tumor volume and degree of heating (9), the hazards of inadequate hyperthermia (5, 8), and the importance of tumor population kinetics (7).

Currently available methods for heating tumors leave much to be desired, and considerable advances in technology are required if hyperthermia is to become a routine therapy modality in the clinic. In addition, it has become apparent that, to destroy the majority of common human solid tumors, temperatures in excess of 42° will be required (13). At such temperatures, the differential effect of heat on cancer cells is lost (6), and the value of hyperthermia for destroying tumors is determined by the extent to which normal surrounding tissues can be spared or protected from damage. In this paper, we report data on the *in vivo* destruction of solid VX2 rabbit tumors up to 22 ml in size by single short exposure to a low-power RF² field. The specially designed RF machine enabled intratumor temperatures of 50° to be achieved rapidly and controllably without damage to overlying skin.

MATERIALS AND METHODS

The VX2 tumor is a highly malignant, anaplastic, squamous cell carcinoma that developed as a result of malignant change in the cells of a Shope virus-induced skin papilloma in a domestic rabbit (20). The carcinoma was transferred by serial inoculation of 1 million cells 1 cm deep into the thigh muscles of male New Zealand White rabbits weighing 2.0 to 2.5 kg (25). The cells gave rise to a palpable tumor by 3 weeks after injection. Untreated rabbits died within a further 7 weeks [mean survival time, 72 ± 7 (S.D.) days] with local and distant spread of the disease.

Tumor volumes were calculated from caliper measurements made in the anteroposterior, lateral, and vertical planes of the limb. Allowance was made for the normal tissues of each animal (25).

¹ The work was supported by the North of England Council of the Cancer Research Campaign.

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² The abbreviation used is: RF, radiofrequency.

The tumors were treated at 3 to 4 weeks after inoculation (volume, 10 to 20 ml), when the tumors were relatively nonnecrotic and metastases were already present in the regional, iliac, and paraaortic lymph nodes and in the lungs.

In Vivo Studies

Tumor Heating. The equipment for heating tumors by RF currents was designed by 1 of us (W. B. W.) and is available from Critical Systems Inc., Palo Alto, Calif. Chart 1 shows the basic circuitry of the apparatus, which was developed using phantoms of 0.9% NaCl gel as heating models. A generator circuit with a crystal-controlled oscillator operating at 13.56 MHz and a resonator circuit tuned to the generator circuit constitute the basic electronics. The generator and output circuits are linked by capacitive coupling with 2 flat paddle electrodes. A condenser or electrostatic field is achieved by placing these electrodes on diametrically opposite sides of the tumor mass, the tissue thus becoming part of the dielectric of a capacitor. The paddles, which may be of various shapes and sizes according to the dimensions of the tumor being heated, are coated with a thin film of low-loss dielectric. This minimizes heating of the paddles, and the low frequencies associated with switching transients are presented with a high capacitive reactance, minimizing the possibility of electric shock. The insulating paddle handles contain compensating inductance coils tuned to the circuit. These neutralize the capacitive reactance between the electrodes and the tissue and, hence, maximize the heating component of the current in the tissue. A further improvement in coupling to the electrodes is represented by a "jumper" connection between the outer sheaths of the coaxial paddle cables. This provides for optimal current flow between the heating paddles and ensures that the ends of the cable sheaths are grounded with respect to the RF voltage. For avoidance of the necessity of adding adjustable tuning controls to the equipment and for provision for accurate RF current measurements with panel meters, the length of the coaxial cables from the generator to the electrodes was kept well below 0.1 wavelength (λ). A frequency of 13.56 MHz represents a λ of 22 m, and the coaxial cables were therefore kept at a maximal length of 1 m, taking into account the velocity of propagation in coaxial cable, which is typically 60 to 70% of that in free space.

The generator has a 2-channel output with a total power capacity of 320 watts. When the L channel is used, the R

channel is fed into a 50-ohm dummy load. Low currents are obtained by incorporating a 6-db attenuator into the L-channel output. RF power is measured in amps, and the potential drop across the tissue (volts) by a high-impedance vacuum tube voltmeter across the paddles. For high stability of the power control and a constant current for variations in spacing between the electrodes, a d.c. inverse feedback loop was developed to interconnect the output RF and the master crystal oscillator. An overload relay automatically shuts off the generator output at an RF current of 2.2 amps, providing a necessary safety factor.

Temperature Measurement. Temperatures were monitored by thermistor probes and a 12-channel direct-reading electric thermometer (Model 3GID; Light Laboratories, Brighton, England) with a scale range of 36–56° and an accuracy of $\pm 0.1^\circ$. For intratumor, intraabdominal, or s.c. temperature measurement, the probes used were 3- or 5-cm-long, needle-type IH, 0.8 mm in diameter. The thermistor bead was located approximately 1 mm proximal to the needle bevel and was suitably insulated from the steel needle barrel. Polythene-covered Type IMR sensors were used for rectal recordings. The probes were individually earthed to the Light meter, and the cables were shielded to minimize interference from the RF field. An Ivac digital thermometer (Ivac Corporation, San Diego, Calif.) with a rectal probe was also used to survey body temperature.

Tissue temperatures were also measured by thermocouple sensors. These were needle probes (5 cm long, 0.8 mm in diameter) with copper-constantan miniature thermocouples incorporated into the needle just proximal to its bevel. The wires of the thermocouple junction were insulated from each other and from the needle barrel. Thick copper and constantan leads with the same thermoelectric constants as the sensor wires were individually insulated to avoid the formation of spurious junctions, and the cables were shielded to the base of the needle. The probes were used in conjunction with 3 different recording systems, 2 digital meters and an analog system. Both digital readout meters had a scale range of 0–199.9°, 1 being a 5-channel instrument (Trendicator 400; Doric Instruments, San Diego, Calif.) and the other being a single-channel recorder (Versacon digital indicator, Model 4FO 120AF; Gulton Europe Ltd., Brighton, Sussex, England). The analog register was a Cambridge potentiometer (Type 44228). All 3 temperature monitors had an accuracy of $\pm 0.1^\circ$.

The electric thermometers were standardized against a

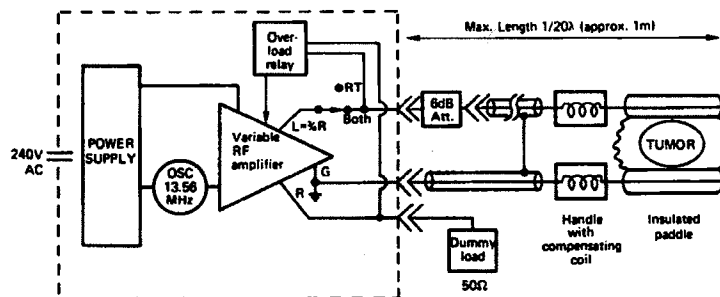


Chart 1. Basic circuitry of RF heating system. Components within the broken square constitute the RF machine (A) in Fig. 1. The option switch shows both outputs of the generator, the L channel heating a tumor via the 6-db attenuator and the R channel being fed into a 50-ohm dummy load.

mercury-in-glass thermometer of the National Standards Laboratory, Hemel Hempstead, Hertfordshire, England, and the probes and meters were checked for "drift" before each day's experiments.

Heating Procedure. All experiments were performed in a temperature-controlled room at 25°. The rabbits were anesthetized with i.m. Hypnorm, 0.5 ml/kg [Hypnorm, Janssen Pharmaceutica; Fentanyl base (0.2 mg/ml), Fluanisone (10 mg/ml), Crown Chemical Company Ltd., Lamberhurst, Kent, England].

After the rear limb(s) were shaved with electric clippers, final traces of hair were removed by a 5-min application of hair-remover cream. The probes were then inserted. With multiple sensors in the tumor, the needles were placed in the proximal and distal poles, medial and lateral poles, or center of the mass. The needles were inserted in different planes of the tumor relative to each other to encompass, as far as possible, the whole mass of the tumor. Skin temperature was monitored by a needle introduced s.c. between the tumor and heating paddle. All probes were positioned at right angles to the RF field, ensuring that there was no contact between paddle and needle. One surface of each paddle was coated lightly with conducting electrode jelly (Cam Creme; Kent Cambridge Medical Ltd.), and the 2 paddles were applied to diametrically opposite sides of the tumor, with the rabbit lying on its side (Fig. 1). The tumor was heated as part of a cylinder of tissue, the cylinder ends being the paddles, the paddle diameter selected and the cylinder volume heated being governed by the size of the tumor.

In Vitro Studies

For incubation or manometry, the tumor was removed from the animal under sterile conditions, cut into pieces, and washed with Rinaldini saline (32) containing penicillin

(100 units/ml), streptomycin (100 µg/ml), and Mycostatin (100 units/ml). Tumor material was then cut into thin fragments (less than 1 cu mm) with scalpel blades and kept at 4° until required. For incubation, approximately 5 g tissue were placed in 5 ml Waymouth Medium MB 752/1 supplemented with 10% pooled human AB serum, in 20-ml Universal glass bottles with airtight caps. After gassing with 5% CO₂ in air, the bottles were incubated in a shaking water bath at controlled temperature.

Warburg Manometry. Traditional Warburg manometry with 100- to 200-mg tumor slices was used in the metabolic studies. Respiration was measured as O₂ uptake in a Krebs-Ringer phosphate buffer, pH 7.4, containing sodium succinate (0.013 M); the center well of the flask contained 0.2 ml 10% KOH, and the gas phase was air. Anaerobic glycolysis was measured as CO₂ production in a Krebs-Ringer:bicarbonate:phosphate solution (24), pH 7.4, containing glucose (2 g/liter) with a gas phase of 95% N₂:5% CO₂. All observations were performed in duplicate flasks at an incubation temperature of 38°. Results were expressed as µl gas exchanged per 10 mg tumor, dry weight, per hr.

RESULTS

In Vitro Metabolic Studies

Chart 2 details the results of *in vitro* thermal sensitivity assay on the VX2 tumor. Tumor slices were incubated in medium at elevated temperature for 30 min. After 2 washes in warm Rinaldini saline, the slices were subjected to Warburg manometry at 38° over 4 hr. Preincubation at temperatures up to and including 45° had no significant effect on O₂ uptake or CO₂ production by the tumor. After incubation at 47°, respiration was reduced by 50% and glycolysis by 70% compared to values at 38°. Respiration was reduced by 80% and glycolysis by 90% following heating at 50°. Gas exchange was linear after heating for 30 min at the temperatures examined. Total inhibition of O₂ uptake and CO₂ production occurred after 2 hr preincubation at 50°. Following heating at 47° and above, tumor slices (100 to 200 mg) did not take when transplanted into rabbits, whereas slices subjected to lower temperatures did take. Therefore, 47° was chosen as the minimal operational temperature for destruction of the VX2 tumor.

In Vivo Studies

Temperature Measurement. Extensive preliminary investigations revealed that, at low power outputs (up to approximately 6 watts, the power level required to maintain tumor temperature at a minimum of 47°), the analog meters (Light thermistor system and Cambridge thermocouple system) were unaffected by the RF field. When sensors were placed in tumor or normal muscle between the paddles in such a field, the difference in registered temperatures with the RF current on and off did not exceed 0.2°. In contrast, the Doric meter reading was 1.0–1.5° higher than the stable recorder systems in this RF field; the results with the Gulton instrument were unstable and irregular. At the power outputs required in the present work to achieve the required intratu-

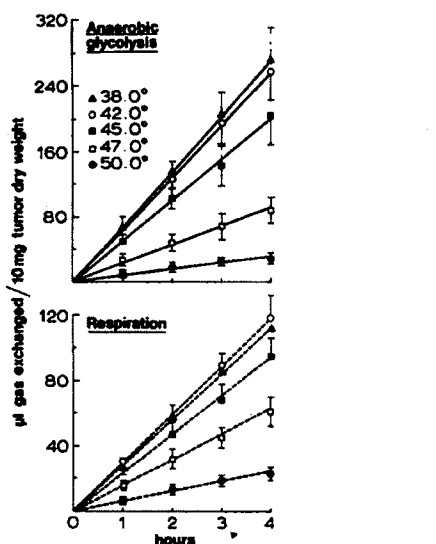


Chart 2. O₂ consumption (---) and anaerobic CO₂ production (—) of VX2 carcinoma slices (100 to 200 mg) over 4 hr in Warburg manometers at 38°. The slices were preincubated for 30 min at 38, 42, 45, 47, or 50°. All manometric observations were performed in duplicate flasks. Each point is the mean ± S.D. from at least 3 different experiments.

mor temperature (10 to 12 watts), only the Cambridge potentiometer readings remained unaffected by the RF field. The Light meter readings were consistently 1.0–1.5° higher and the Doric Trendicator readings were 1.0–3.0° higher in the presence of the RF current than in its absence.

When the RF current applied to a VX2 tumor was switched off, the maximal rate of temperature decrease within the tumor, with the heating paddles removed, was approximately 1.4°/min (measuring the temperature of the highest recording thermistor needle, 50°, over 5 min; Chart 3). When the current was switched off and the paddles remained in position (as during the 30-min heating period), the comparable rate of temperature decrease was approximately 0.9°/min. Since the response time of the thermistors is 4 sec and 4 probe temperatures can be read within 15 sec, it is apparent that the thermistors are capable of accurately recording the temperatures used. In practice, during heating the difference in temperature measured by an individual sensor 5 to 6 and 15 to 20 sec after the RF field ceased was usually less than 0.2°. This indicates that the localized heating effect due to concentration of the RF field in the region of the needle was small. The validity of the temperatures recorded was confirmed by simultaneous temperature measurements with the Cambridge potentiometer system and by insertion of thermistors, which had been equilibrated at 45°, into tumors immediately after terminating the RF field and reading of the stable temperature 5 to 6 sec later.

Temperature was therefore routinely monitored with thermistor sensors, recordings being made with the RF field switched off. The multichannel Light thermistor system was much more convenient in operation than the Cambridge potentiometer.

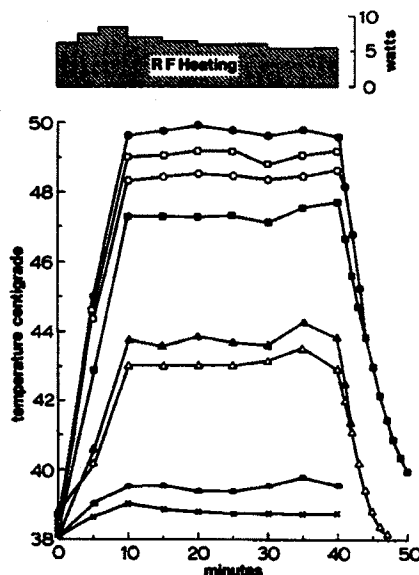


Chart 3. Temperature profiles for a rabbit with a 16-ml VX2 tumor growing in the left thigh muscles. The tumor was maintained at 47° for 30 min by RF heating; the heating paddles were then removed for the cooling curve measurements. Thermistor sensors were placed at 4 positions in different planes within the tumor mass (●, ○, ■, □), s.c. beneath the heating paddles (▲, △), in normal muscle 1 cm proximal to the heating paddles (▲, △), and in the rectum (×).

Tumor Heating. Chart 3 indicates the potential of the RF equipment for achieving selective high-temperature destruction of large tumors. VX2 carcinomas were usually heated to 47° using less than 10 watts power achieved gradually over 10 min. The electrical resistance of the tissue decreased with time, enabling the intratumor temperature to be maintained by a substantially reduced current (0.6 to 1.1 amps). Temperature patterns within large tumors (15 to 22 ml) indicated less homogeneous heating than in smaller tumors. At 47°, a temperature differential of 2–3° was usually observed between multiple sensors in a large tumor. Skin temperature over the tumor remained at 43–44° throughout the 30-min heating period. The temperature of normal muscle situated 1 cm outside the RF field did not increase above 40°, and the rabbits' rectal temperature remained within the normal range. Tumor and skin temperatures returned rapidly to normal when the RF field was switched off.

Ten VX2 carcinomas, ranging in volume from 12 to 22 ml, have been treated by RF heating at 47–50° for 30 min, 7 of the animals being cured. The tumors regressed in 6 to 8 weeks. In the other 3 rabbits, following heating there was paralysis of the tumor-bearing limb, and the animals had to be "put down" within 3 to 4 weeks of therapy. During this period, there was no increase in tumor volume.

A series of experiments was performed to investigate the pattern of heating of tumor and normal tissue exposed to the same RF field. The tumor-bearing limb and the animal's normal leg were lightly strapped together at the ankle. A cylinder of tissue of a diameter adequate to include the tumor and adjacent muscle of the tumor-bearing limb was heated, with 1 paddle applied over the tumor area and the other applied to the lateral side of the normal leg. Passage of the current between the legs was facilitated by coating the apposing skin surfaces in the field with conducting cream.

Chart 4 shows the temperature profiles obtained in 1 of 4 similar experiments in which the VX2 carcinoma was heated at 47° for 30 min as part of a tissue cylinder that included both rear legs of the rabbit. The muscle adjacent to the tumor and the muscle in the normal limb maintained a temperature of 44° during the heating period. The temperature of muscle in the tumor-bearing limb outside the RF field followed the rectal temperature and increased from 37.5–41°. In these experiments, the temperature of the skin beneath the 2 paddles was maintained at 43.4–44.5°, the skin over the VX2 being at a higher level than that over the normal muscle. In the carcinoma, the plateau heating temperature was attained more rapidly than in the muscle within the RF field, but there was little difference in the slope of the cooling curves of the 2 tissues.

Table 1 records the detailed electrical measurements obtained with RF heating of normal legs and tumor-bearing limbs of the rabbit in experiments similar to that depicted in Chart 4. The tissues were heated to 47° and maintained at this temperature for 30 min. From the mean amperage (*I*) and mean potential difference (*V*) across the tissue cylinder during the 30-min heating period, the tissue resistance (*V/I*) was calculated. The geometry of the heated tissues is indicated by the diameter and separation of the paddles, and the range of amps and volts required to maintain these cylinders of different volume at temperature is given. The

presence of tumor in the muscle did not significantly alter the electrical resistance of the leg(s). The resistance of the rabbit limb at 44° (Chart 4) was similar to the resistance at 47°.

DISCUSSION

Approaches to the local heat treatment of tumors have occupied the attention of experimentalists since the turn of the century. A myriad of techniques has been employed, ranging from the crude application of fomentations (16, 31) and the convenience of water bath immersion (4, 12) to high-frequency electric currents (19, 29, 37) and the expertise of regional perfusion (3). Electrically induced hyperthermia

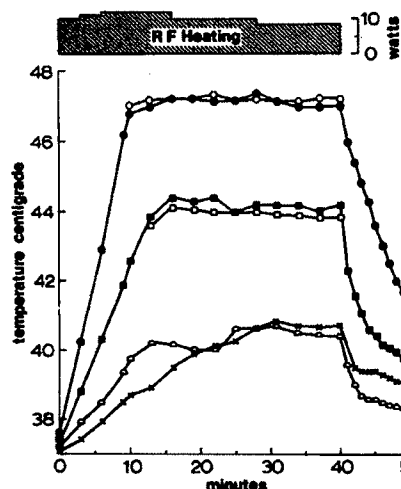


Chart 4. Simultaneously recorded temperatures and cooling curve patterns for a cylinder of tissue comprising a normal and a tumor-bearing (left) rear limb of a rabbit. The legs were held together by a nonrestrictive tape around the ankles. One heating paddle (6-cm diameter) was positioned to include in the RF field the tumor (volume, 11.3 ml) and part of the leg muscle surrounding the tumor, with the other similar paddle applied to the opposite limb at a distance of 5.2 cm. Temperature was recorded in the tumor maintained at 47° (●, ○) and within the RF field in muscle of the tumor-bearing leg 1.5 cm from the tumor margin (□) and in muscle of the opposite normal limb (■). Outside the RF field, temperature was monitored in muscle of the left leg 1 cm proximal to the heating paddle (△) and in the rectum (×). Skin temperature recorded beneath the paddles was maintained within the range of 43.3–44.5° over the 30-min heating period (10 to 40 min). At 40 min, the RF current was terminated, the heating paddles were removed, and temperatures were recorded at 1-min intervals to construct the cooling curves.

has encompassed the electromagnetic spectrum, from the use of conventional diathermy frequencies in the region of 1 MHz (λ 300 m) to treat the Crocker sarcoma in mice (28) to frequencies approaching the maximum that can be used to give depth of penetration, namely microwaves at 3000 MHz (λ 10 cm), for treating transplantable rat hepatoma 223 (2).

With electrical heating, workers who have measured intratumor temperature have achieved temperatures of 45–50° *in vivo* easily and rapidly. However, an intractable problem has been the high resistance presented by the skin to the passage of electric currents and the differential heat conductance of bone and fatty tissue on the one hand and muscle and other deep tissues with high water content (e.g., liver, kidney) on the other (34).

Previous workers have encountered varying degrees of damage to the skin and internal organs and inhomogeneous tumor heating. These factors have led to inadequate tumor heating and low-percentage cure rates, even with small s.c. tumors. Westermarck (37) treated Flexner-Jobling and Jensen tumors of 0.3 to 5.8 ml growing s.c. in the flank of rats. A high-frequency current (unspecified) at 0.1 to 0.4 amp and "low" tension across the electrodes was employed. The active electrode applied to the tumor was 15 to 20 sq cm in size, and a larger 100-sq cm ground electrode was positioned on the opposite side of the animal's body. Westermarck used a large needle, 12 cm long and 2 mm in diameter with an iron constantan thermocouple at its tip, and a galvanometer with an accuracy of 0.25° to measure temperature. Most of the tumors heated were less than 2.5 ml in volume, and at tumor temperatures over 46° superficial to deep skin damage occurred commonly, but the incidence is difficult to evaluate since it was most marked when there was skin infiltration by the tumor. Even with tumors of 0.5 ml volume, Westermarck reported animal death, during or shortly after treatment, from heat stroke or extensive coagulation necrosis of internal organs. Johnson (19) designed his own transmitter to heat Walker 256 and Jensen tumors growing s.c. in rats. The tumors (volume, 0.5 to 2.0 ml) were gripped between the electrodes and subjected to short radio waves (88 MHz, λ 3.4 m), giving temperatures of 43.5–50° for up to 1 hr, exposure time being decreased as the temperature increased. Temperature within the RF field was monitored by a Cambridge copper-constantan thermocouple and galvanometer similar to that used in the present work. Generally, damage to healthy skin did not occur at

Table 1

Protocol for maintaining normal and tumor-bearing limbs of the rabbit at 47° for 30 min by RF heating

The tissue was heated as a cylinder between 2 paddles of equal diameter applied to opposite sides of the leg(s). Paddle diameter was chosen to be greater than or equal to the thickness of the limb(s); with tumor-bearing legs, the paddle diameter was always greater than the tumor diameter measured in the same plane. Tumor volumes ranged from 11.3 to 22.0 ml.

Limbs examined	Volts (V)	Amps (I)	Resistance (ohms = V/I)	Paddle diame-ter (cm)	Paddle sep- aration (cm)
One leg (4)*	4–7.6	0.25–0.5	14.9 ± 2.2 ^a	2.5 or 3.0	2.3–2.8
Both legs (4)	6–10.3	0.4–0.7	14.3 ± 1.4	5.0	4.5–5.2
One leg with tumor (10)	7.5–14.0	0.61–1.1	13.9 ± 1.9	5.0 or 6.0	4.3–5.2
Both legs, 1 with tumor (4)	5.0–9.6	0.4–0.85	13.5 ± 2.4	5.0 or 6.0	5.0–6.2

* Numbers in parentheses, number of different animals examined in each group.

^a Mean ± S.D.

treatment temperatures below 47°. As found by Westermarck (37), even after heating in the 46–50° range, a considerable number of tumors were unrestrained or regressed only temporarily after treatment. The margins of the tumor were not as hot as the central portions, but there was no differential heating of the tumor with respect to surrounding tissues, which were protected only at peripheral tumor temperatures below 47° (19). Cater *et al.* (2) treated hepatoma 223 tumors (volume, 1 to 2 ml) in the thigh muscles of rats for 5 to 10 min with RF microwaves at 3000 MHz (λ 10 cm). Tumor temperature during heating was continuously monitored by a constantan wire soldered into the tip of a stainless-steel dental needle to form a thermocouple. It was sometimes difficult to control the temperature of the tumor. At 47°, this was reflected in edema of the foot or areas of thrombosis in the tumor or overlying tissues; at this temperature, exposure times beyond 10 min incurred the danger of limb destruction. No tumor cures were obtained by these workers. Overgaard and Overgaard (29) treated small (up to 0.2 ml) s.c. mammary carcinomas in C₃H mice by short-wave diathermy at 27.12 MHz (λ 11 m). These workers stated that "it was possible to obtain a temperature in the central part of the tumor of up to 46 to 47°, apparently without damaging the skin or the underlying tissue." A copper-constantan thermocouple inside an air-filled pointed glass tube was used to measure tissue temperature in the RF field. However, an optimal tumor cure rate of 25% was obtained by heating the tumors at 43° for 60 min, and these workers found little advantage in using intratumor temperatures in excess of 43° (29).

The strain of VX2 tumor used in the present work was not sensitive to 42°, in contrast to the tumor previously investigated by us (10, 25). However, the method and rate of spread of the cancer were similar to that already reported, as was the disappearance of metastases associated with regression of the primary tumor following curative hyperthermia (10, 25). This systemic response of tumor elsewhere in the host to local hyperthermic destruction of tumor [the so-called "abscopal" response (15)] is a striking feature of successful local heating and has now been recorded in several animal tumor systems and in man (see Refs. 5 and 6 for a review). Marked inhibition of metabolism *in vitro* was not obtained with the current tumor at temperatures below 47° (Chart 2). Several reports have indicated that inhibition of tumor respiration and/or glycolysis *in vitro* can be used as a guide to the temperature required to cause tumor regression in the host (see Ref. 13 for a review). Recent work has also shown that, for irreversible damage to tumor cells by heat, inhibition of respiration need not be total (11). The validity of 47° as the minimal curative temperature to maintain during heating of these large tumors was supported by the findings that tumor slices heated at <47° grew when transplanted into rabbits and that VX2 carcinomas heated at 46.5° did not regress but killed the host. The close agreement between the tumor-destructive temperatures in these *in vitro-in vivo* experiments further supports the validity of the *in vivo* temperature measurements obtained in the present work.

RF currents in the short-wave diathermy range (10 to 100 MHz) have proved notoriously difficult to quantitate as to

dose because the relationships between machine-generated power and power consumed by heated tissue are complex (34, 35). However, it has been repeatedly demonstrated that the depth of penetration and the temperature distribution within the tissue is critically dependent upon the contact obtained between the heated part and the electrodes and on the geometry of the electrodes, as well as the frequency employed (26, 34, 35). In the present experiments, efficient contact between the heating paddles and the skin, from which all traces of hair must be removed, was crucial, as was the length of the tissue cylinder heated in relation to its diameter. Further important factors involved in easily controlled tumor heating and the maintenance of a temperature differential between skin and deeper tissues may be the use of the lower end of the short-wave diathermy spectrum (13.56 MHz), with its greater depth of penetration in tissue (18) and minimal current spread beyond the electrodes; the employment of the electrostatic (condenser) field technique rather than the more common inductive field technique of applying the current; and the incorporation of compensating coils in the paddle handles.

An RF apparatus operating at 13.56 MHz and apparently similar in construction to the currently described machine was used recently by LeVein *et al.* (21) to treat tumors in animals and man. The authors claim that temperatures of 46–50° were achieved in solid human tumors of the lung, colon, and kidney and in cancers of the head and neck; the method of temperature measurement is not mentioned. One to 9 treatments, each lasting 30 min, led to extensive tumor necrosis and regression in a series of 21 patients. Mouse ascites carcinoma and Brown-Pearce tumors in rabbits were raised to a temperature of "7 to 9C above that of the surrounding tissue. These tumors were rapidly and completely necrosed." The report is totally bereft of detail in relation to the animal work, but the human patients received 1 to 4 watts/sq cm over the 30-min treatment period. The comparable RF power required to maintain the VX2 tumors at 47–50° for 30 min was 0.25 to 0.45 watt/sq cm (assuming LeVein's figures were calculated per sq cm of the live electrode only). The higher power values reported by LeVein *et al.* may reflect the greater heat required for internal tumors in a larger host (humans) and/or differences in the geometry of the electrodes and heating configuration (no details of this or of tumor size were reported by these workers).

LeVein *et al.* (21) also measured blood flow through a small number of human tumors *in vitro* and found it to be only 1 to 15% of that through the normal adjacent tissue. These authors attribute the selective RF heating of tumors to this decreased blood flow, which is inhibitory to rapid heat dissipation. The present results of exposing VX2 tumor and normal rabbit muscle to the same RF field (Chart 4; Table 1) may support this view. Although the resistance offered to an electric current by an electrolyte-dielectric composite like a living body cannot be explained merely by Ohm's Law, tissue resistance is a major factor in high-frequency heating, which in general follows the ordinary laws of electric heating (27, 34, 37). Since the tissue resistance, as measured under standardized conditions, was not significantly affected by the presence of the tumor (Table 1),

the differential heating of tumor and muscle (Chart 4) may reflect a poorer blood flow through the tumor and an inability to cope as effectively with the increased heat load.

Although the electrical heating of tumors is not new, the potential of this approach remains largely unexploited due to the problems of quantifying the dose, standardizing the field configuration, and recording temperature accurately. However, the scope available and the effect of small alterations in diathermy technique are well illustrated by the recent results of Mendecki et al. (22). By means of a specially designed applicator and microwaves at 2450 MHz, these workers obtained a 100% cure rate in small (0.2 ml) s.c. mammary adenocarcinomas in mice after heating at 43° for 45 min on 2 occasions. Temperatures in the 10-watt microwave field were monitored by thermistor and thermocouple probes. In previous work, only a 25% regression rate was obtained by short-wave (27.12 MHz) heating of this tumor at 43° (29).

The problems of temperature measurement in high-frequency electric fields due to the liability of metallic sensors to high-frequency pick-up have been discussed by previous investigators (2, 19, 29, 37). It is apparent from the results of these workers that, under defined conditions, adequately constructed thermocouples and an analog recording system can be used to obtain a reliable measure of tissue temperature. Our experience with the digital recorders indicates that the major problem in temperature measurement in an RF field up to 10 to 12 watts resides in processing the signal from the thermosensor rather than in self-heating of the probe.

The present approach represents a considerable advance in technology for local hyperthermic treatment of the VX2 carcinoma compared with previous methods (10, 25). Only the humane killing of 3 animals with paralysis of the treated limb prevented a cure rate in excess of 70%. Limb paralysis was not specifically associated with RF heating, since it has occurred in a similar manner and with a similar time course after other forms of limb heating (e.g., water bath immersion) used in this laboratory for treating rabbits.

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REFERENCES

- Braun, J., and Hahn, G. M. Enhanced Cell Killing by Bleomycin and 43° Hyperthermia and the Inhibition of Recovery from Potentially Lethal Damage. *Cancer Res.*, 35: 2921-2927, 1975.
- Cater, D. B., Silver, I. A., and Watkinson, D. A. Combined Therapy with 220KV Roentgen and 10 cm Microwave Heating in Rat Hepatoma. *Acta Radiol. Therapy Phys. Biol.*, 2: 321-336, 1964.
- Cavaliere, R., Ciocatto, E. C., Giovannella, B. C., Heidelberger, C., Johnson, R. O., Margottini, M., Mondovi, B., Moricca, G., and Rossi-Fanelli, A. Selective Heat Sensitivity of Cancer Cells. *Cancer*, 20: 1351-1381, 1967.
- Criie, G. Heat as an Adjunct to the Treatment of Cancer: Experimental Studies. *Cleveland Clinic Quart.*, 28: 75-89, 1961.
- Dickson, J. A. Hazards and Potentiators of Hyperthermia. *International Symposium on Treatment of Cancer by Hyperthermia and Radiation*, Washington, D. C., 1975, pp. 134-150. Baltimore, Md.: American College of Radiology Press, 1976.
- Dickson, J. A. The Effects of Hyperthermia in Animal Tumor Systems. *Recent Results Cancer Res.*, 59: 43-111, 1977.
- Dickson, J. A., and Calderwood, S. K. *In Vivo* Hyperthermia (42°C) of the Yoshida Tumor Induces Entry of Non-Proliferating Cells into Cycle. *Nature*, 263: 772-774, 1976.
- Dickson, J. A., and Ellis, H. A. Stimulation of Tumor Cell Dissemination by Raised Temperature (42°C) in Rats with Transplanted Yoshida Tumors. *Nature*, 248: 354-358, 1974.
- Dickson, J. A., and Ellis, H. A. The Influence of Tumor Volume and the Degree of Heating on the Response of the Solid Yoshida Sarcoma to Hyperthermia (40-42°). *Cancer Res.*, 36: 1188-1195, 1976.
- Dickson, J. A., and Muckle, D. S. Total Body Hyperthermia versus Primary Tumor Hyperthermia in the Treatment of the Rabbit VX2 Carcinoma. *Cancer Res.*, 32: 1916-1923, 1972.
- Dickson, J. A., and Oswald, B. E. The Sensitivity of a Malignant Cell Line to Hyperthermia (42°C) at Low Intracellular pH. *Brit. J. Cancer*, 34: 262-271, 1976.
- Dickson, J. A., and Suzangar, M. *In Vivo-in Vitro* Studies on the Susceptibility of the Solid Yoshida Sarcoma to Drugs and Hyperthermia (42°). *Cancer Res.*, 34: 1263-1274, 1974.
- Dickson, J. A., and Suzangar, M. A Predictive *In Vitro* Assay for the Sensitivity of Human Solid Tumors to Hyperthermia (42°C) and Its Value in Patient Management. *Clin. Oncol.*, 2: 141-155, 1976.
- Dickson, J. A., and Suzangar, M. The *In Vitro* Response of Human Tumors to Cytotoxic Drugs and Hyperthermia (42°C) and Its Relevance to Clinical Oncology. In: M. Balls and M. Monnickendam (eds.), *Organ Culture in Biomedical Research*, pp. 417-461. Cambridge, London, and New York: Cambridge University Press, 1976.
- Goldenberg, D. M., and Langner, M. Direct and Abscopal Antitumor Action of Local Hyperthermia. *Z. Naturforsch.*, 26b: 359-361, 1971.
- Gottschalk, S. Zur Behandlung des Ulcerierenden Inoperablen Cervixcarcinoms. *Zentr. Gynakol.*, 79-80, 1899.
- Jasiewicz, M. L., and Dickson, J. A. Potentiation of the Destructive Effect of Heat (42°C) on Synchronized Cancer Cells in Culture by Cell Specific Antiserum. *J. Thermal Biol.*, 1: 221-225, 1976.
- Johnson, C. C., and Guy, A. W. Non-Ionizing Electromagnetic Wave Effects in Biological Materials and Systems. *Proc. IEEE*, 60: 692-718, 1972.
- Johnson, H. J. The Action of Short Radio Waves on Tissues. III. A Comparison of the Thermal Sensitivities of Transplantable Tumors *In Vivo* and *In Vitro*. *Am. J. Cancer*, 38: 533-550, 1940.
- Kidd, J. G., and Rous, P. A Transplantable Rabbit Carcinoma Originating in a Virus-Induced Papilloma and Containing the Virus in Masked or Altered Form. *J. Exptl. Med.*, 71: 813-838, 1940.
- LeVeen, H. H., Wapnick, S., Piccone, V., Falk, G., and Ahmed, N. Tumor Eradication by Radiofrequency Therapy. *J. Am. Med. Assoc.*, 235: 2198-2200, 1976.
- Mendecki, J., Friedenthal, E., and Botstein, C. Effects of Microwave-Induced Local Hyperthermia on Mammary Carcinoma in C3H Mice. *Cancer Res.*, 36: 2113-2114, 1976.
- Mondovi, B., Agro, A. F., Rotilio, G., Strom, R., Moricca, G., and Rossi-Fanelli, A. The Biochemical Mechanism of Selective Heat Sensitivity of Cancer Cells. II. Studies on Nucleic Acids and Protein Synthesis. *European J. Cancer*, 5: 137-146, 1969.
- Mondovi, B., Strom, R., Rotilio, G., Agro, A. F., Cavaliere, R., and Rossi-Fanelli, A. The Biochemical Mechanism of Selective Heat Sensitivity of Cancer Cells. I. Studies on Cellular Respiration. *European J. Cancer*, 5: 129-136, 1969.
- Muckle, D. S., and Dickson, J. A. The Selective Inhibitory Effect of Hyperthermia on the Metabolism and Growth of Malignant Cells. *Brit. J. Cancer*, 25: 771-778, 1971.
- Oliphant, W. D. High Frequency Therapy. Part III. Electrode Theory and Design. *Electron. Eng.*, 16: 252-255, 1943.
- Oliphant, W. D. High Frequency Therapy. Part IV. The Electric Field in a Dielectric. *Electron. Eng.*, 16: 296-299, 1943.
- Overgaard, K., and Okkels, H. Über den Einfluss der Wärmebehandlung auf Woods Sarkom. *Strahlentherapie*, 68: 587-619, 1940.
- Overgaard, K., and Overgaard, J. Investigations on the Possibility of a Thermic Tumor Therapy. I. Short-wave Treatment of a Transplanted Isologous Mouse Mammary Carcinoma. *European J. Cancer*, 8: 65-78, 1972.
- Overgaard, K., and Overgaard, J. Radiation Sensitizing Effects of Heat. *Acta Radiol. Therapy Phys. Biol.*, 13: 501-511, 1974.
- Percy, J. F. Heat in the Treatment of Carcinomas of the Uterus. *Surg. Gynecol. Obstet.*, 22: 77-79, 1916.
- Rinaldini, L. M. J. An Improved Method for the Isolation and Cultivation of Embryonic Cells. *Exptl. Cell Res.*, 16: 477-505, 1959.
- Robinson, J. E., Wizenberg, M. J., and McCready, W. A. Combined Hyperthermia and Radiation Suggest an Alternative to Heavy Particle

- Therapy for Reduced Oxygen Enhancement Ratios. *Nature*, 251: 521, 1974.
34. Schwan, H. P. Biophysics of Diathermy. In: S. Licht (ed.), *Therapeutic Heat and Cold*, Ed. 2, pp. 63-125. Baltimore: Waverly Press, 1965.
 35. Scott, B. O. Short Wave Diathermy. In: S. Licht (ed.), *Therapeutic Heat and Cold*, Ed. 2, pp. 279-309. Baltimore: Waverly Press, 1965.
 36. Suit, H. D., and Shwayder, M. Hyperthermia: Potential as an Anti-tumor Agent. *Cancer*, 34: 122-129, 1974.
 37. Westermarck, N. The Effect of Heat upon Rat Tumors. *Scand. Arch. Physiol.*, 52: 257-322, 1927.

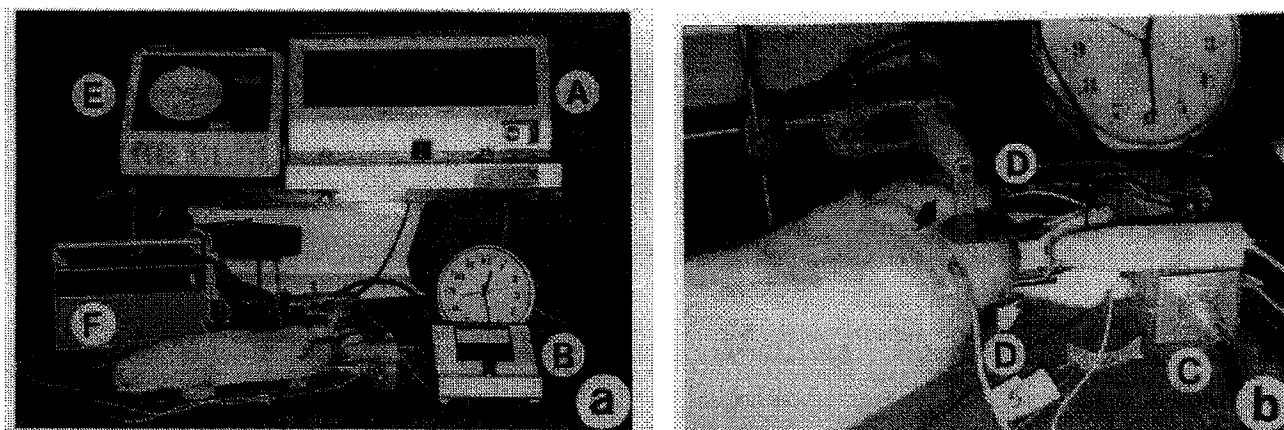


Fig. 1. Experimental arrangement for treatment of rabbit VX2 limb tumor by RF heating (a) and close-up of electrode configuration (b). The RF machine (A) measures 48 x 48 x 28 cm and is portable. Current generated by the machine, measured as amps, is applied to the tumor by 2 heating paddles (arrows) placed on diametrically opposite sides of the tumor mass. Potential drop across the paddles (volts) is measured by means of a high-impedance vacuum tube voltmeter (B). Low currents are obtained by inserting a resistance (attenuator, C) between the RF machine and the live paddle. Temperatures in the tumor and host are continuously monitored by multiple sensors (D) inserted at right angles to the RF field and coupled to a 12-channel electric thermometer (E) and a digital readout instrument (F).